

Rapid Biochemical Presumptive Test For Gonorrheal Urethritis in the Male

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DURING the past few years a series of biochemical paper tests have been developed for rapid, presumptive laboratory identification of various colony growths on solid bacteria media. The reagents in the various test papers are designed to show a color reaction when exposed to specific enzymes produced by certain bacteria. One of these biochemical tests using Pathotec—Co paper (A) involves identification of cytochrome oxidase, an enzyme characteristic of the *Neisseria* and *Pseudomonas* species. According to Ewing and Johnson (1), species of the following genera also give a positive cytochrome oxidase test: *Aeromonas*, *Vibrio*, *Alcaligenes*, and some *Flavobacteria*. The specific biochemical reaction involved in the cytochrome oxidase test is shown in figure 1.

In the presence of cytochrome oxidase, the reagent zone of the test paper turns a bright blue.

The test paper has two zones of the impregnated chemicals. As used in the laboratory a concentrated colony of unidentified bacterial growth is applied by wire loop to one zone. To the other zone a known cytochrome oxidase positive organism is applied for comparison of color reaction.

The possibility of putting this laboratory test into clinical use prompted the trials to be described. If sufficiently sensitive, the test might prove a valuable one for screening suspected gonorrhea in clinical practices in which stand-

ard laboratory procedures are not readily available.

Since the male with urethral discharge can be most readily diagnosed with accuracy, initial trials were limited to men admitted for diagnosis in the venereal disease clinics of Seattle, Wash., and San Diego, Calif. This report details our experience with the biochemical test during the first 3 months of 1965.

Materials and Methods

Both clinics carried out their studies independently with only minor variations in procedure. In each clinic, all men complaining of urethral discharge, regardless of previous history or presence of other symptoms, were tested on a consecutive basis. The patient was instructed to express as much urethral discharge as possible to the meatal orifice of the penis. The clinician then held the reagent zone of the test paper close enough to collect material directly on both sides of the paper. This procedure permits a maximum amount of fluid discharge to come in contact with the reagents of the paper strip. The test paper was immediately placed in a small plastic test tube and covered with a tight-fitting plastic cap. A minimum of 10 minutes was allowed to elapse before the clinician read and recorded the result. In San Diego, the clinician sent the tube to the laboratory for a second reading and recording.

The urethral smear (gram-stained) was used by both clinics to confirm the diagnosis of gonorrhea. In San Diego, a culture of urethral discharge was also routinely done on each patient. In Seattle, isolation of *Neisseria gonorrhoeae* by culture was routinely performed only on those patients whose slides were re-

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ported as negative or doubtful. About a third of the patients with positive smears were also, however, subjected to urine cultures for confirmation.

Culture techniques in the two laboratories varied. The San Diego clinic used a small amount of peptone starch fluid as a transport medium for cotton applicators containing urethral discharge. Upon delivery in the laboratory, the culture was transferred to Thayer-Martin medium (2) for incubation and identification. The culture technique in Seattle consisted of streaking sediment from a centrifuged, freshly voided urine specimen on chocolate agar medium. After incubation in CO₂ for 24 hours, suspicious colonies were examined by fluorescent antibody microscopy for identification.

In San Diego, one clinician (co-author) conducted the clinical testing, whereas the Seattle clinic used all physicians who regularly staff the clinic. Physicians in both clinics read and recorded results of the biochemical test before receiving laboratory reports on smears and cultures.

It is important that sufficient liquid discharge be transferred directly to the test paper without use of cotton swabs. The liquid portion of the discharge, rather than the cellular portion, probably contains the cytochrome oxidase enzyme in the quantity necessary to produce the color reaction in the paper strip. Placing the paper strips in test tubes prevents rapid drying

of secretions and allows the slower reactions to materialize. These slower reactions may be associated with continued production of enzyme until the gonococcal organisms die. The time required for the color reaction to appear varied from 30 seconds to approximately 5 minutes.

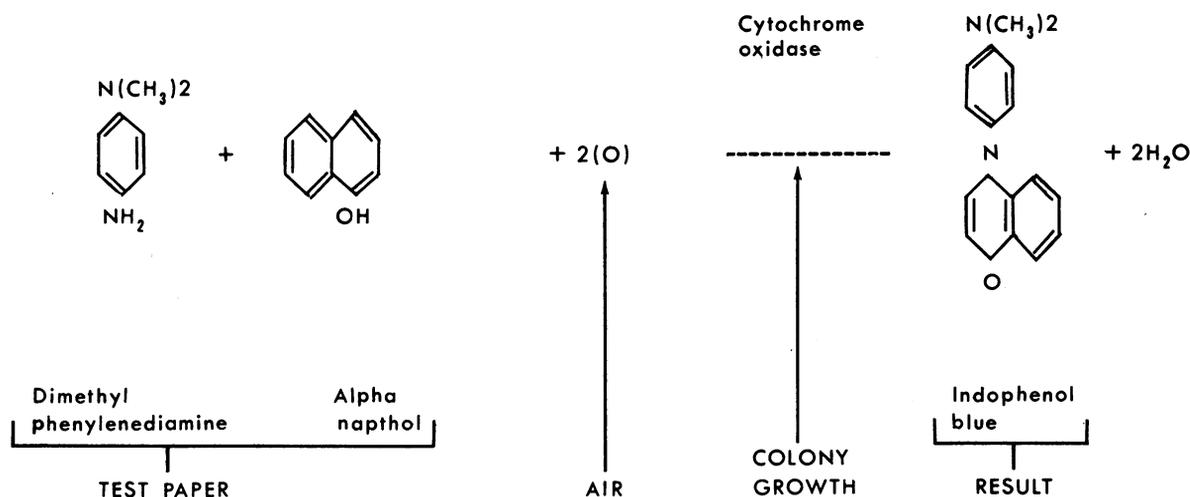
When the purulent discharge is greenish and positive for gonorrhea, the reagent zone may turn bluish-green rather than bright blue. Usually under these circumstances, a periphery zone of brighter blue will be noted beyond the bluish-green area. Color reactions appear to be stable for several weeks. Test papers should be refrigerated when not in use to prevent deterioration. The photograph (fig. 2) shows three positive reactions and one negative reaction to the biochemical test. The first three tubes, left to right, contain paper strips used in testing three patients with proven gonorrheal urethritis. The fourth represents results obtained in nongonorrheal urethritis.

Results

A total of 288 patients were studied during the 3-month period. In table 1 findings from each clinic are compared as to results of smears, cultures, and biochemical tests. Correlation of the biochemical test with the various categories of bacteriological findings are also given.

Smartt (3) has recently pointed out that a negative smear or culture by itself may not be reliable in ruling out gonorrhea. In this study the possibility of gonorrhea was ruled out only

Figure 1. Biochemical reaction involved in the cytochrome oxidase test



if both tests were negative on each patient. It is accepted by most clinicians that the gram stain is reliable in diagnosing acute gonorrhoea in the male if the smear is positive.

Table 2 shows the combined data for the two clinics with the percentage of correlation. If the physician has a patient with a positive biochemical test, he can probably rely on at least 78 percent accuracy. A lack of sufficient patients with nongonorrhoeal urethritis has prevented us from obtaining an adequate measure of specificity.

Discussion

We observed that the majority of the 26 false-negative reactors had only scanty urethral discharge. Sufficient material for a smear could be

obtained only by inserting a sterile cotton applicator into the distal portion of the urethra. Some of these men had urinated 10 to 15 minutes before the examination. If the patients had been selected on the basis of observable amounts of discharge rather than by history of discharge, the sensitivity of the test might well have been higher than 78 percent.

Further investigation is needed to explain the presence of cytochrome oxidase enzyme in urethral discharge from false-positive reactors. The most likely cause, theoretically, is infection with enzyme-producing bacteria other than *N. gonorrhoeae*. In Seattle, 3 of the 15 false-positive reactors were rechecked for the presence of *Pseudomonas* infection. None was found. The possibility of male patients having gonor-

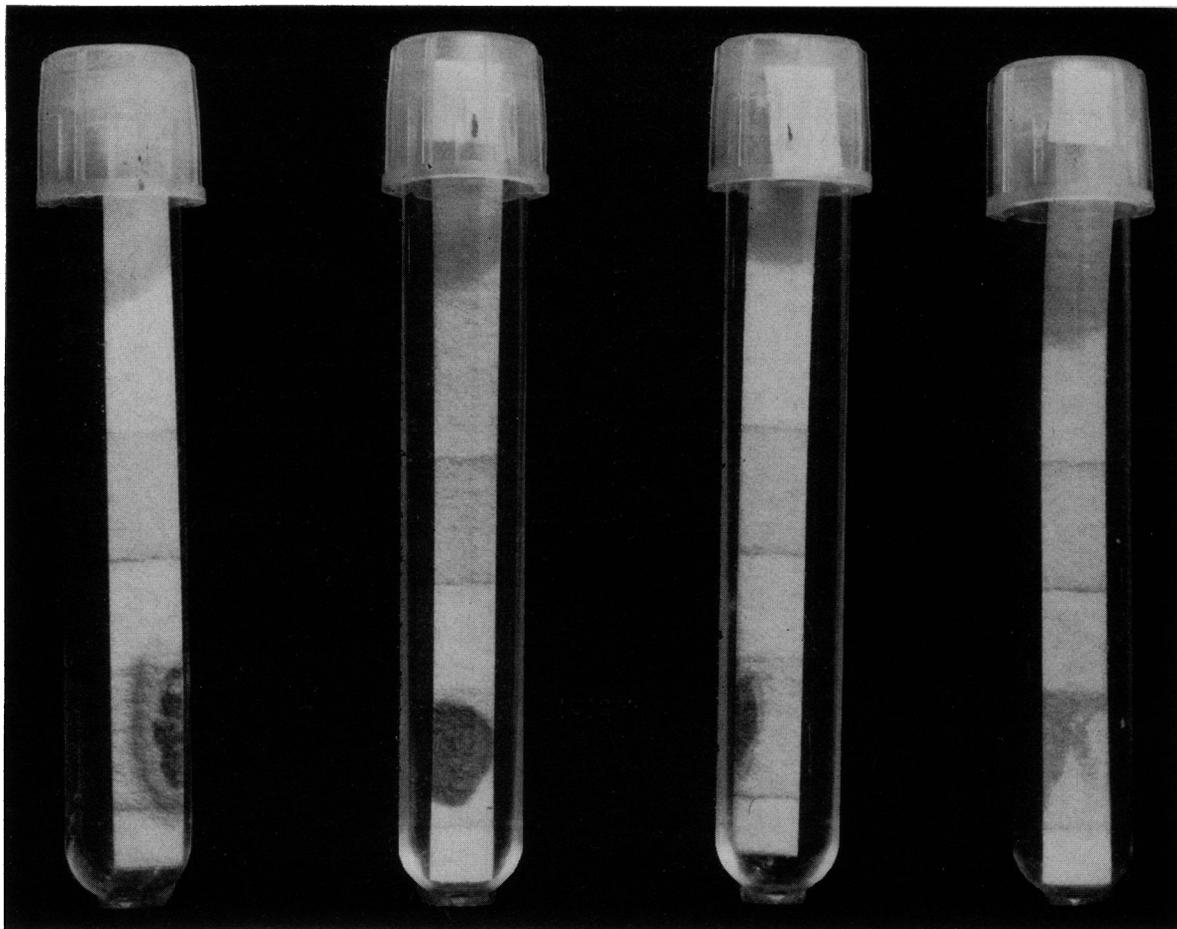


Figure 2. Positive and negative reactions to the cytochrome oxidase test. The first three tubes, left to right, contain paper strips used to test three patients with proven gonorrhoeal urethritis; the fourth tube represents results in nongonorrhoeal urethritis

Table 1. Results of biochemical, smear, and culture tests on male clinic patients with suspected gonorrheal urethritis, Seattle-King County, Wash., and San Diego, Calif.

Biochemical test group and number of patients	Test results		
	Bio-chemical	Smear	Culture
Seattle clinic (N=122)			
Positive test:			
34.....	+	+	+
48.....	+	+	Not cultured
2.....	+	+	-
2.....	+	-	+
15.....	+	-	-
Negative test:			
9.....	-	-	-
9.....	-	+	Not cultured
2.....	-	+	+
1.....	-	-	+
San Diego clinic (N=166)			
Positive test:			
112.....	+	+	+
9.....	+	+	-
5.....	+	-	+
22.....	+	-	-
Negative test:			
4.....	-	-	-
14.....	-	+	+

rhea despite negative smears and cultures must also be considered. At least 5 of the 15 patients having false-positive biochemical tests in Seattle had a clinical record of repeated previous gonorrheal infections. Two of these five patients had had positive laboratory findings of gonorrhea within the previous month. In view of current problems with penicillin-resistant strains of gonococci (4), it is possible that some patients represented treatment failures and that cytochrome oxidase enzyme of gonococcal origin was detectable regardless of smear and culture results.

Possibly, patients seen in a public venereal

disease clinic differ significantly from the spectrum of male patients treated by the private practitioner. The patient of the public clinic is likely to be a young adult male who suspects he has been exposed to venereal disease. Hospitals and practitioners, on the other hand, may see many patients with urethral discharge from other etiological causes. The specificity of the biochemical test needs to be tested under these conditions. Only in this manner can other specific etiological bacteria responsible for false-positive biochemical reactions be determined.

Significant epidemiologic control of gonorrhea has been an elusive goal of public health workers for many years. The true incidence of the disease in the United States is unknown, but it has been estimated that 1.5 million new cases develop each year (5). A recent national survey (5) indicated that perhaps 75 percent of these cases are being seen and treated in the private physician's office. An estimated 10 percent are being reported to health agencies. This limited reporting implies that contact interviewing and new casefinding cannot be effectively pursued by public health workers. Until more accurate diagnostic tests are available to confirm gonococcal disease in the female, increased epidemiologic efforts in interviewing male patients treated by private practitioners seems to offer the most promise in reducing the reservoir of infection in the community.

The private physician is understandably reluctant to report gonorrhea and permit patients to be interviewed unless he has bacteriological confirmation of disease. If the physician can be offered a sensitive and rapid screening test, he might well prefer to have the public health laboratory perform such bacteriological tests and might allow interviews with his patient. Sensitivity levels achieved with the biochemical test justify additional clinical investigation to determine its potential in the epidemiologic control of gonorrhea. If further clinical trials by others substantiate our results, the test could conceivably fulfill the practitioner's need.

Many areas of the world lack adequate laboratory facilities and trained professional staffs. A simple, rapid, and fairly accurate test could be of inestimable value in combating gonorrhea. Paramedical personnel could easily be trained to screen large population groups.

Table 2. Comparison of biochemical test with final bacteriological diagnosis in 288 male patients with suspected gonorrheal urethritis, Seattle-King County, Wash., and San Diego, Calif., 1965

Group	Total patients	Biochemical, bacteriological tests agree		Biochemical, bacteriological tests disagree			
		Number	Percent	False negative		False positive	
				Number	Percent	Number	Percent
Seattle-King County-----	122	95	77.9	12	9.8	15	12.3
San Diego-----	166	130	78.3	14	8.4	22	13.3
Combined-----	288	225	78.1	26	9.0	37	12.9

Our results also suggest the possibility of using the biochemical test in followup of patients who appear to represent treatment failures.

Summary

An initial trial of a rapid biochemical test for the presumptive clinical diagnosis of gonorrheal urethritis in the male was carried out on 288 male patients attending the venereal disease clinics of San Diego, Calif., and Seattle, Wash. Both clinics reported a 78 percent positive agreement between the biochemical and the standard bacteriological tests. This result at present represents the degree of sensitivity of the biochemical test. It seems likely, however, that if patients had been selected on the basis of a more clinically obvious discharge, the sensitivity level would have been even higher than that obtained in the trial.

The specificity level of the test should be investigated in a population group other than that attending a public venereal disease clinic.

Present sensitivity levels justify further clinical investigation to assess the potential use-

fulness of this biochemical test in the epidemiologic control of gonorrhea.

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- (4) Thayer, J. D., and Moore, M. B., Jr.: Gonorrhea: present knowledge, research and control efforts. *Med Clin N Amer* 48: 755-765, May 1964.
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EQUIPMENT REFERENCE

- (A) PathoTec—Co paper, General Diagnostics Division of Warner-Chilcott Laboratories, Morris Plains, N.J.